

## Nutritional performance of *Tupinambis merianae* lizards fed with corn starch as source of energy

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**Abstract.** Efficiency in processing complex carbohydrates as a source of energy was studied in *Tupinambis merianae* lizards. Four isoproteic and isoenergetic diets in which different percentages of corn starch substituted fat (0, 10, 20 and 30 dry matter in the diet) were provided. Even though consumption was similar in all diets, growth and feeding conversion rates decreased significantly with corn starch supplies of 10% and more. At the end of the trial, pancreatic alpha-amylase activity showed correlated increases, yet these were insufficient to compensate corn starch supplies. Results suggest that *Tupinambis merianae* lizards have a restricted omnivorous capacity. Therefore, diet formulation for these lizards should exclude high molecular weight carbohydrates.

**Keywords.** *Tupinambis merianae*, omnivorous capacity, energy supply, growth, feeding conversion.

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### INTRODUCTION

The genus *Tupinambis* (Squamata: Teiidae) comprises a group of large size lizards exclusive to the South American plain, east of the Andes (Presch, 1973). The southernmost species, *T. merianae* (formerly *T. teguixin*) and *T. rufescens* (Cei and Scolaro, 1982), constituted a traditional resource of aboriginal communities as a source of meat, fat and leather (Donadio and Gallardo, 1984; Norman, 1987). In the last decades, an intense and sustained exploitation of natural populations has taken place to commercialize these species skin, employed in the design of luxury items (Fitzgerald et al., 1991). Between 1975 and 1986, extraction in Argentina was reported to have reached over 16 million skins (Chardonnet et al., 2002). This situation had international impact, causing the inclusion of these species in Appendix II of CITES. Although skin harvest is currently regulated, the authorized extraction quota is still very large: one million skins per year (Basso et al., 2005). Fortunately, captive breeding programs (Mercolli and Yanosky, 1990; Noriega et al., 1996) supported by a better understanding of these the saurian biology allows foreseeing a more rational use without affecting natural populations.

From a nutritional point of view, one of the most significant advances consisted in the design of a hatchery diet, which resulted in growth rates considerably higher than those referred to in previous literature (Vega Parry and Manes, 2000, 2004). This advance was mainly based on the reinterpretation of dietary habits in *T. merianae* and *T. rufescens* as essentially carnivorous (Vega Parry et al., 2000; Manes et al., 2007). It is pertinent to note the existence of ontogenetic changes in *Tupinambis* dietary habit associated with changes in tooth structure, but always within a carnivore regime (from insects to more bulky and active preys) (Presch, 1974; Dessem, 1985).

These results do not exclude, however, the possibility of a limited omnivorous capacity in these animals. In fact, a true omnivorous status has been reported for *Tupinambis* by some authors, based on the presence of vegetal material in their digestive tract (Milstead, 1961; Donadío and Gallardo, 1984; Mercolli and Yanosky, 1994). In this respect, we find it useful from a practical point of view, as of digestive physiology, to analyse the efficiency of these animals in processing complex carbohydrates as a source of energy.

This study evaluates growth responses and pancreatic alpha-amylase activity induction in *T. merianae* lizards fed on corn starch, as a possible alternative integrated diet to improve their production in captivity, thus avoiding the culling of specimens from the wild.

## MATERIALS AND METHODS

### *Animals and experimental conditions*

The study was conducted in spring, during the highest feeding rate period (November-December) using 24 ten-month-old *T. merianae* juveniles born in captivity and fed on a hatchery diet (Vega Parry and Manes, 2000). These were selected from a population of approximately 200 individuals from 12 different clutches. At this stage the gender of the specimens could not be distinguished.

They were treated orally with 0.5 ml/kg of levamisole hydrochloride to preclude incidental parasites, and were placed separately in outdoor individual cages (0.9 m long  $\times$  0.6 m wide  $\times$  0.6 m high), supplied with shelter, sunny and shaded spaces, a trough and a drinking dish. Troughs consisted of one-opening receptacles having a plastic cover to avoid direct sunlight and food dispersal.

### *Diets*

Four diets were compared. One was taken as the control diet (diet A), and it consisted of bovine lean meat and refined fat, supplemented with 5% tricalcium phosphate, 0.75% avian vitamin-mineral supplement (Micromix-Biofarma), 0.75% common salt and 0.25% butyl-hydroxytoluene (BHT) on a dry matter basis. The others were prepared by replacing the fat matter in isoenergetic proportions, with 10 (diet B), 20 (diet C) and 30% (Diet D) corn starch (lipids: 39.34 kJ/g; corn starch: 17.58 kJ/g). (Hafez and Dyer, 1972; INRA, 1986). Table 1 shows ingredients, protein and energy levels for each diet.

### *Feeding process*

To ensure complete intake of the different diets, a consumption test was performed prior to the experiment, using the control diet (A). From the obtained value (see Results), followed the dry matter calculation of the remaining diets (B, C and D) for similar protein and energy delivery.

**Table 1.** *Tupinambis merianae* daily supplies per 100 g of live weight.

Diets (*)	Meat (g dry matter) / Protein (g) (**)	Fat (g) / gross energy (kJ) (***)	Corn starch (g) / gross energy (kJ)	Total gross energy (kJ)	Energy to protein relationship
A (0% starch)	1.39 / 1.15	0.69 / 27.43	— / —	54.62	47.50
B (10% starch)	1.39 / 1.15	0.58 / 23.06	0.25 / 4.39	55.18	47.98
C (20% starch)	1.39 / 1.15	0.46 / 18.29	0.50 / 8.79	54.27	47.19
D (30% starch)	1.39 / 1.15	0.35 / 13.92	0.75 / 13.18	54.29	47.20

(\*) Supplemented with tricalcium phosphate (0.1 g); avian vitamin-mineral supplement (0.015 g); common salt (0.015 g) and butil-hidroxi-toluene (0.005 g).

(\*\*)  $N \times 6.25$  (correction factor).

(\*\*\*) Values correspond to the incorporated fat, plus 4% corresponding to interstitial muscular fat.

Food was provided daily at around 1000 h, at the beginning of the diel activity period (emergence from shelters). Adjustments to diet followed weight and snout-vent length (SVL) increase in each individual, recorded every 5-7 days. Growth was estimated in terms of body mass to the nearest 0.1 g, and SVL to the nearest 0.1 cm increase. Conversion efficiency was calculated as the proportion between body mass gain and dry mass eaten. (Staton et al., 1990; Manes et al., 2007).

#### *Experimental design and statistical analysis*

After 2 days fasting, 24 animals with similar weights were selected. Each animal was assigned a diet at random and then was placed in outdoor individual cages, thus conforming 4 groups of 6 individuals. In order to evaluate if initial live weights were similar in every treatment, an analysis of variance was conducted. Results showed no significant differences in initial weights among treatments ( $P = 0.99$ ).

Since the individuals constituted a random sample and only four diets were tested, a mixed model approach was used in order to analyze data (Pinheiro and Bates, 2000). The variables evaluated in the fixed part of the model were treatment and concomitant variables, and a random effect was included for the animals. In the case of total weight gain and feed conversion, initial live weights were used as concomitant variables. In the case of total SVL gain, initial live weights and initial SVL were used as concomitant variables. Prior to the analyses, it was verified that a linear relation existed between the concomitant variables and the responses. Significant components for the fixed part of the model were chosen by backward elimination. Alternative models were compared by means of likelihood ratio tests (Pinheiro and Bates, 2000).

Treatments means were compared to the control by means of Dunnet's contrasts. Statistical analyses were performed in R (R Development Core Team, 2008). The R package used was nlme (Pinheiro et al., 2008).

#### *Diet chemical and other analysis*

Protein, fat, ash and moisture contents of the diets were determined by Wendee's proximal analysis (AOAC, 1980). Diet gross energy was calculated using protein, lipid and corn starch energetic values, i.e. 23.65, 39.34 and 17.58 kJ/g respectively (Hafez and Dyer, 1972; INRA, 1986).

At the end of the assays, three individuals fed on each diet were euthanized with an overdose (60 mg/kg) of sodium pentobarbital (AVMA, 2007) injected into the caudal vein. The pancreas was removed and stored at  $-20^{\circ}\text{C}$ . The enzyme extracts were prepared by homogenizing 150 to 300 mg of pancreatic tissue in 1.5 ml of 0.2 M Na-phosphate buffer, pH 7 at  $4^{\circ}\text{C}$ . The extracts were centrifuged at 10000 rpm, recovering the supernatant which constituted the crude enzymatic preparation. Amylase activity was determined by a kinetic method (GT lab) in diluted samples (1/100). Proteins were quantified by the method of Bradford (1976). An analysis of variance test was performed on data and Dunnett's post hoc test was applied. Analysis was performed in R (R Development Core Team, 2008).

## RESULTS

### *Consumption determination*

The average daily consumption recorded for the 24 individuals, fed on control diet (diet A) during 15 days was  $2.5 \pm 0.08$  g of dry matter per 100 g of bodymass. This value was considered to provide similar protein and energy supplies in the different treatments, without food rejections.

### *Growth and feeding responses*

The 4 diets were supplied during a 30 days-assay in which the animals were effectively fed for 26 days. No food was provided on days when the weather was cold or rainy, since animals remained in the shelters. Complete acceptance of food without rejections at the end of each day indicated a similar protein and energy intake in the different diets.

The progressive replacement of fat with corn starch in diets resulted in a clear decrease in growth, as reflected both in mass as in SVL (Table 2). Growth decrease was non-significant only for diet A and in terms of SVL. Correspondingly, feeding conversion rate declined significantly, beginning with a 10% corn starch substitution (Table 2). Although animals doubled their initial weight, still they did not show evidences of sexual dimorphism.

### *Alpha-amylase activity*

*T. merianae* juveniles fed on corn starch had their physiologic compensation, increasing their pancreatic alpha amylase activity levels. At the end of the trial, it was observed that activity levels produced by diets with 20 and 30% starch were significantly higher than those resulting from the control diet (50 and 123% respectively, Table 3).

## DISCUSSION

Carbohydrate-free control diet (A) resulted in major growth and best food conversion rate in *T. merianae* young individuals with respect to other diets with different level of

**Table 2.** Growth and feeding conversion of *Tupinambis merianae* juveniles fed on different starch levels during 30 days (mean  $\pm$  SE).

Item	Diet A (0% starch)	Diet B (10% starch)	Diet C (20% starch)	Diet D (30% starch)
Initial live weight (g)	667.03 $\pm$ 71.47	657.95 $\pm$ 77.63	660.41 $\pm$ 80.01	679.73 $\pm$ 87.99
Final live weight (g)	1463.20 $\pm$ 129.79	1363.33 $\pm$ 130.81	1285.13 $\pm$ 134.20	1214.78 $\pm$ 135.53
Total weight gain (g)	796.17 $\pm$ 58.90	705.38 $\pm$ 53.99*	624.72 $\pm$ 55.38**	535.05 $\pm$ 51.71**
Initial SVL (cm)	26.75 $\pm$ 1.11	26.75 $\pm$ 0.86	26.33 $\pm$ 0.83	26.41 $\pm$ 1.24
Final SVL (cm)	33.83 $\pm$ 1.17	32.91 $\pm$ 1.15	31.91 $\pm$ 1.11	31.75 $\pm$ 1.44
Total SVL gain (cm)	7.08 $\pm$ 0.30	6.17 $\pm$ 0.54	5.58 $\pm$ 0.36**	5.33 $\pm$ 0.25**
Feed conversion: weight gain (g) / ingested dry matter (g)	1.68 $\pm$ 0.05	1.46 $\pm$ 0.06*	1.24 $\pm$ 0.04**	1.02 $\pm$ 0.03**

(\*, \*\*) Mean differences of treatments versus control corresponding to Dunnet's test ( $P < 0.05$  and  $P < 0.01$ ).

**Table 3.** *Tupinambis merianae* pancreatic alpha-amylase activity responses to corn starch feeding.

Diets	Alpha amylase units / mg pancreatic protein (*)
A (0% starch)	582.12 $\pm$ 41.21
B (10% starch)	635.53 $\pm$ 41.94
C (20% starch)	868.83 $\pm$ 48.62 **
D (30% starch)	1273.39 $\pm$ 196.56 **

(\*) alpha amylase units: amount of enzyme needed to convert 1  $\mu$ mol of substrate per minute (2-chlorine-4-nitrophenyl-alpha-D-maltotriose).

(\*\*) Mean differences  $\pm$  SE of treatments versus control corresponding to Dunnet's test ( $P < 0.01$ ).

carbohydrate. After a 30-day trial experience, individuals supplied with carbohydrate free diet exhibited a 2.2-fold increase in weight and a 1.3-fold increase in LHC (Table 2).

The replacement in the diet of only 17% of the fat with its energy equivalent in corn starch (diet B) was enough to produce a delay in lizard growth, which intensified with major substitutions. As protein and energy intake was similar in all treatments, and no food rejections were recorded, results can be directly attributed to the difficulty for these lizards to digest starch.

The quantity of this ingredient in *Tupinambis* diet is markedly inferior to that used in farm diets for other animals (i.e., diets for poultry, pigs and rabbits), which include a great quantity of cereal flour rich in amylaceous carbohydrates (Scott et al., 1973; Osman, 1982; INRA, 1986). It is also lower when compared with quantities used in rats omnivorous diet models (Thornburg et al., 1987) and in human diets (Gray, 1992).

The restricted use of starch by *T. merianae* appears to indicate a feature common to all carnivores (Pieper and Pteffer, 1980; Staton et al., 1990; Kienzle, 1993a). It has also

been suggested that faulty carbohydrate digestion by these animals can indirectly hinder the appropriate assimilation of proteins (Kienzle, 1994; Refstie et al., 2000; Aleixo et al., 2002; Zoran, 2002).

Various physiological studies have considered pancreatic alpha-amylase enzyme in numerous species as indicating omnivorous capacity, on the grounds of its regulation in relation to carbohydrate levels in diets (Jain, 1976; Brannon, 1990; Kienzle, 1993b; Topping et al., 1997; Chowdhury et al., 2000). The present studies show higher pancreatic alpha-amylase enzyme activity levels in *T. merianae*, on account of starch contents in diet. This value doubles with the maximum starch supply (diet C, 30% starch), in contrast to a scarce increase in this activity in a strictly carnivorous animal, such as the cat (Kienzle, 1993a). Nonetheless, the value reported for *T. merianae* is still notably inferior to the one recorded for various omnivorous species, with increases by up to 500% with comparable energy substitutions (Brannon, 1990).

Thus as in the case of other carnivores, *T. merianae* exhibits a limited physiologic adaptation to a typically omnivorous diet (Skoczylas, 1978; Kienzle, 1993a), which is consistent with the reduced length of the large intestine and the lack of structures necessary for fermentative digestion in this species (Vega Parry et al., 2000).

Even if the influence of species sexual dimorphism cannot be discarded (Donadio and Gallardo, 1984; Mercolli and Yanosky, 1990; Fitzgerald et al., 1991; Noriega et al., 1996) in the present results, the homogeneity and juvenile status of the studied animals, expressing final weights of 60% of the one necessary to reach sexual maturity (unpubl. data), suggests that this influence is negligible.

In conclusion, previous references to *Tupinambis* as an omnivorous species, based on discoveries of vegetal material in the digestive tract of animals living in their natural habitats (Milstead, 1961; Donadio and Gallardo, 1984; Mercolli and Yanosky, 1994), should be carefully reconsidered. In this sense, the fact that these animals are keen on sweet fruit rich in monosaccharides (Donadio and Gallardo, 1984), and the fact that a high proportion of them are found as vegetal residues (Milstead, 1961; Mercolli and Yanosky, 1994), seem to point at an opportunistic intake of monosaccharides rather than a real omnivorous capacity in this species.

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